

What is claimed is:

1. A method of classifying cells into subpopulations using cell classifying data, the method comprising:
 - 5 receiving image data;
 - analyzing said image data to identify object areas in the image data;
 - analyzing said image data, on the basis of said identified object areas, to determine, for at least one selected first cell, one or more measurements;
 - deriving a first parameter set for said at least one selected first cell, the first
 - 10 parameter set comprising at least one of said one or more measurements;
 - classifying a first set of cells, the process of classifying the first set of cells including classifying said at least one selected first cell into a subpopulation and storing first identifying data indicating the subpopulation into which said at least one selected first cell has been classified;
 - 15 deriving cell classifying data for use in classifying a second set cells into subpopulations from said first parameter set and said first identifying data, and
 - classifying a second set of cells into subpopulations on the basis of one or more measurements taken for cells in the second set of cells, by use of said cell classifying data.
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2. The method of claim 1, wherein said first identifying data is cell cycle phase classifying data.
3. The method of claim 2, wherein classifying said second set of cells comprises
- 25 comparing the measurements for cells in the second set with the cell cycle phase classifying data derived from classification of the first set of cells.
4. The method of claim 1, wherein classifying said second set of cells comprises calculating a statistical likelihood of each cell in the second set being a member of a
- 30 subpopulation.
5. The method of claim 1, wherein a plurality of measurements are taken, and weighted in statistical importance.

6. The method of claim 1, wherein applying said cell classifying data to a second set of cells further comprises generating cell cycle phase population data indicative of the relative sizes of said plurality of sub-populations in the selected cells.
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7. The method of claim 1, further comprising performing the method with image data from a plurality of wells containing cells, the plurality of wells containing different test compounds.
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8. The method of claim 1, wherein said object areas are identified using a process arranged to select both nuclear and cytoplasmic areas of a cell.
9. The method of claim 1, wherein said object areas include, for a selected cell, a first type of object area and a second type of object area, and wherein said one or more measurements include a first measurement determined using said first type of object area and a second measurement determined using said second type of object area.
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10. The method of claim 9, wherein said first type of object area is identified using a process arranged to select a predominantly nuclear area of a cell.
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11. The method of claim 9, wherein said second type of object area is identified using a process arranged to select a predominantly cytoplasmic area of a cell.
12. The method of claim 1, wherein said one or more measurements include, for a selected cell, a first measurement determined using an identified object area and a second measurement determined using an identified object area.
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13. The method of claim 12, wherein said first and second measurements are determined using the same identified object area.
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14. The method of claim 1, wherein cells of said first and second sets of cells comprise at least a first luminescent reporter, wherein said step of receiving image data comprises receiving first image data created by detecting radiation emitted by said first

luminescent reporter, and wherein said step of analyzing said image data to determine one or more measurements comprises analyzing said first image data.

15. The method of claim 14, wherein said step of analyzing said image data to identify object areas comprises analyzing said first image data.
16. The method of claim 14, wherein at least one cell in said first and second sets of cells further comprises a second luminescent reporter indicative of the location of a sub-cellular component in a cell.
17. The method of claim 16, wherein said step of receiving image data comprises:
- a) receiving first image data created by detecting radiation emitted by said first luminescent reporter; and
 - b) receiving second image data created by detecting radiation emitted by said second luminescent reporter,
- wherein said step of analyzing said image data to identify object areas comprises analyzing said second image data, and
- wherein said step of analyzing said image data to determine one or more measurements comprises analyzing said first image data.
18. The method of claim 14, wherein said one or more measurements include a measurement of a cytoplasmic luminescence signal intensity, taken in an area generally corresponding to a cytoplasmic component of a selected cell.
19. The method of claim 14, wherein said one or more measurements include a measurement of a nuclear luminescence signal intensity, taken in an area generally corresponding to a nuclear component of a selected cell.
20. The method of claim 14, wherein said step of analyzing said image data to identify object areas comprises analyzing said first image data.

21. The method of claim 1, wherein said cell classifying data is used in conjunction with an algorithm to classify a selected cell into a selected first one of a plurality of sub-populations of cells.
- 5 22. The method of claim 21, wherein the algorithm takes into account a plurality of measurements in a parameter set.
23. The method of claim 1, wherein said one or more measurements include one or more measurements selected from the group consisting of:
- 10 *I*, a parameter relating to an average signal intensity within an identified object area;
F, a parameter relating to a fraction of pixels that deviate more than a given amount from an average signal intensity within an identified object area;
H, a parameter relating to the number of pixels with a signal intensity below a given threshold within an identified object area;
- 15 *A*, a parameter relating to a ratio between major and minor axes of an elliptical outline corresponding to an identified object area;
R, a parameter relating to a maximum width of an identified object area;
L, a parameter relating to an average width of an identified object area;
C, a parameter relating to signal texture within an identified object area;
- 20 *M*, a parameter relating to margination in an identified object area.
24. The method of claim 1, wherein a second parameter set is derived from said one or more measurements taken for the second set of cells.
- 25 25. The method of claim 24, further comprising the modeling of a parameter set as a feature vector in an *n*-dimensional feature space, where *n* is equal to the number of parameters.
26. The method of claim 25, wherein a feature vector representing said second parameter set and a feature vector representing said first parameter set occupy the same feature space.
- 30 27. The method of claim 26, wherein a distance is calculated between the feature vectors.

28. The method of claim 27, wherein the distance between the feature vectors is indicative of the classification of the feature vector representing the second parameter set.
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29. The method of claim 25, wherein a cell represented by a feature vector representing the second parameter set is classified according to a calculation of probability.
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30. The method of claim 29, wherein the calculation of probability comprises calculating the likelihood that the cell represented by said feature vector representing the second parameter set is in the same subpopulation as a cell represented by a feature vector representing the first parameter set, the calculation being based on the dimensions of the feature vectors.
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31. The method of claim 26, wherein a neural network is applied to classify the cell represented by a feature vector representing the second parameter set with respect to the feature vector representing the first parameter set.
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32. The method of claim 1, wherein said cells comprise a nucleic acid reporter construct, preferably a DNA construct, comprising a nucleic acid sequence encoding a detectable live-cell reporter molecule operably linked to and under the control of:
- i) at least one cell cycle phase-specific expression control element, and
 - ii) a destruction control element.
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33. Apparatus arranged to perform the method of claim 1.
34. Computer software arranged to perform the method of claim 1.
35. A data carrier storing the computer software of claim 34.